

PAPERS READ.

BACTERIOLOGICAL OBSERVATIONS MADE AT THE
LITTLE BAY COAST HOSPITAL.

BY DR. OSCAR KATZ.

It was not alone on account of my desire to obtain pure-cultures of the bacillus of typhoid fever (*Bacillus typhi abdominalis*, Eberth-Gaffky), with the view of having them for some intended experiments on this micro-organism (1), but also for the sake of putting forward, by means of the demonstration of its constant occurrence in typhoid fever, fresh proofs of the etiological meaning of this microbe in a country where such experiments have not yet been made known, that I applied to Dr. H. N. MacLaurin, Medical Adviser to the New South Wales Government, to permit me to make a short stay in the Coast Hospital at Little Bay, a place admirably fitted for carrying out investigations of that kind. I gladly avail myself of this opportunity of tendering Dr. MacLaurin

(1) Among a number of cultures of bacteria in agar-agar which Professor Flugge had been kind enough to send to me at the end of last year, there was also the typhoid-bacillus. But unfortunately in this culture on its arrival life was extinct. In the test-tube it had only little expanded; that it was no more alive may be accounted for by the fact that the tube, together with others, had been sent away (via Bremen) immediately after their having been supplied with culture-material, at a time (end of November), when a formation of spores could not be expected. The bacilli very likely died from want of air, which had no access to the interior of the glass-tubes these having been provided with tightly fitting india-rubber-caps.

my best thanks. During the time of my residence in the above Hospital, Dr. W. Peirce, Medical Superintendent, and Dr. R. W. Young, Resident Medical Officer, rendered me every possible assistance; I have much pleasure in gratefully acknowledging this once more.

My endeavours to cultivate the typhoid-bacilli extended, in the main, to organs of persons who died of typhoid fever, and to dejections from typhoid fever patients.

It is well to say in advance that in order to get and to isolate the micro-parasite in question, I made use both of Koch's plate-process, and Esmarch's method of cultivating in test-tubes. About the former nothing more requires to be mentioned. Of the latter which I took occasion to demonstrate at the last meeting of this Society, April, 1887, a detailed description by Esmarch is given in *Zeitschrift für Hygiene*, Band I., Heft 2, Leipzig, 1886, pp. 293-301; an abstract in Flügge's *Microorganismen*, Leipzig, 1886, p. 656. This method was given the preference later on, because the working after the same is connected with less consumption of time, does not require much apparatus, and if properly done yields quite good results. According to my experience very satisfactory cultivations after this process can be obtained in the following manner. The test-tubes containing the liquefied inoculated gelatine (or agar-agar), which in the commonly used test-tubes should not be more than about 6 ccm.—I always employed an 8 p.c. gelatine—are first supplied at the top as well as the bottom, with tightly fitting caps of indiarubber. (Thus the tubes when laid on a level surface are also nearly level). The solidification of the still liquid gelatine (or agar-agar) takes place by means of iced water or at least sufficiently cold water in an ordinary soup-plate, to the bottom of which the tube in its length is loosely pressed by one hand, and quickly turned round its long axis by the other. In a very short time the inner walls of the test-tube show a thin equal

layer of quite transparent solidified gelatine (or agar-agar). For stick-cultures I used a 6 p.c. nutrient gelatine ; for streak-cultures (on an inclined surface) the same, and occasionally a 1 p.c. nutrient agar-agar.

Now it need scarcely be mentioned that, from the mere behaviour of pure cultures in stick and in streak of the typhoid-bacillus, from the appearance of its colonies in diverse nutritive substances either on macroscopical observation or on being viewed with low-magnifying powers, and then from the image of the individual bacilli out of such cultures or out of organs under high powers of the microscope, an exact inference as to their undoubtedly belonging to the *Bacillus typhi abdominalis* cannot be drawn. With regard to the last-named point I can confirm the statements of others, namely, that the dimensions of the rods are not constant, and that these variations depend in the main on the kind of the nourishing material, out of which cultures of the microbe are microscopically examined.

Even the staining reaction of the typhoid-bacilli which become discoloured after the method of Gram (see Flügge, *Microorganismen*, p. 643, or any book dealing with the methods of investigation in Bacteriology) cannot be any more maintained as being diagnostic of these schizomycetes, as a bacillus isolated by Escherich from the feces of young children, and called by him *Bacterium coli commune* (Flügge, *Microorganismen*, p. 269) exhibits the same peculiarity if treated after Gram's method. The only decisive means, so far as known, enabling us to distinguish typhoid-bacilli from all other bacteria, is rather their characteristic growth on slices of boiled potatoes ; in the repeatedly named work of Flügge full particulars may be had. By subsequent study of the bacilli, however, it has been noticed by several investigators that these do not always grow on the potato-surface in the shape of a coherent, resisting membrane which was considered as typical by Gaffky, the first who worked with pure cultures of the bacilli, but that now and then they also

form loose, somewhat visible vegetations, easily removable from the potato-surface. For such small deviations the quality of the used potatoes is made responsible; but it must also be borne in mind that the least possible quantities of seed material should be taken with which to sow potato-slices. Similar deviations have come under my notice, yet in all such cases it cannot be difficult to arrive at a correct diagnosis, if, besides also all the other features exhibited by typhoid-bacilli, microscopical appearances, characters of cultures, and staining reactions, are duly considered.

Quite recently Fraenkel and Simmonds have furnished some more data as to the cultivation of the typhoid-bacilli on potatoes; these remarks seem to me to be sufficiently worthy of note that I give here a complete translation of them. They say (*Zeitschrift f. Hygiene, Band II., Heft 1, Leipzig, 1887, pp. 140-141*):—
 “. . . . In continuing our investigations into the typhoid-bacillus, other far more important deviations have come under our notice, deviations which in the beginning made the purity of our culture appear doubtful to us. Sometimes when we had inoculated numerous potato-surfaces from one gelatine-culture at the same time, it happened that after three or four days some surfaces showed a quite peculiar appearance, besides other normal-looking potato-surfaces. There was on the surface an easily recognisable, grey, viscous coating, the margins of which were very distinctly visible. At those places which were not sown the potato exhibited a brownish colour, and the older the culture grew, the darker became the colour of the parenchym. No smell whatever was perceptible. On examination the normal-looking potato-surfaces were found to contain magnificent specimens of typhoid-bacilli with preference arranged in pseudo-filaments. The examination of the grey, viscous, easily removable cultures of the rest of the potatoes yielded, as result, the presence of an infinitely larger quantity of bacilli, yet these were so far behind their usual length and thickness that undoubtedly every observer, especially

by comparing the different modes of growth on the different potatoes with one another, would have thought of there being two absolutely distinct micro-organisms. But still a mistake could not have occurred, because one and the same gelatine stick-culture had been used in all cases of that kind for the inoculation of the potato-surfaces ; in fact, any mistake was excluded, as something of those grey cultures being transferred to other potatoes resulted in yielding again ordinary, invisible cultures which microscopically showed again normal bacilli and pseudo-filaments. By and by we learnt to distinguish the different sorts of potato, and were thus able, now and then, to anticipate which ones would show invisible and which ones visible colonies. Thus, by mutual transmissions of cultures of one kind of potato to another kind we could occasionally obtain those cultures. All this proves most certainly that with the *technic* no fault was to be found. We might be permitted to state that in our experiments on animals we worked with the two different looking cultures separately, but the results being always alike in either case we considered later on such a separation as superfluous, and in our subsequent annotations these differences of the culture are no more taken notice of. We want purposely to lay special stress on these striking deviations of the potato-cultures, because, in disregard of this demeanour perhaps many an observer might have arrived at wrong conclusions. By this the value of the potato-culture for the identification of the typhoid-bacillus is by no means lessened, only it is advisable in all such-like cases where a growth corresponding to the one above-described makes its appearance, to transfer the questionable potato-culture to several other potato-surfaces before a decisive opinion should be formed."

Such mal-formations are probably brought about in consequence of the use of bad, watery potatoes ; the quality of a potato which it is intended to take as nutrient soil for the cultivation of typhoid-bacilli or of any kind of micro-organisms, can easily be

found out by the mode of preparation about which I supplied some data in these Proceedings, April, 1887, pp. 187-190.

I would now state the results of my experiments in cultivating the typhoid-bacillus from organs of persons dead of typhoid.

Unfortunately for my purposes, but reflecting no little credit on the mode of treatment in the Hospital, the rate of mortality from typhoid fever was here for the last season, in general, exceptionally small, although the number of cases was by no means insignificant. For this reason I was only able to examine the organs of three corpses. The patients had died during the third week of the complaint; the bacteriological examination of different organs—spleen, liver, mesenteric glands—always took place when these were still fresh. The search for typhoid bacilli yielded positive results in each of the three cases, in so far as there appeared, after less time than is commonly the case, (1) in or on the culture media colonies or vegetations of a micro-organism which was decisively proved by the potato-culture to be the *Bacillus typhi abdominalis* (Eberth-Gaffky). In two of the three cases there was besides this no other micro-organism found; in the third case where merely a puncture of the liver was possible, in addition to the numerous colonies of the typhoid-organism a few sulphur-yellow colonies of *Sarcina* developed. Whether these were attributable to some unintended contamination (the whole manipulation was done rather in a hurry) or not, I was unable to trace; in fact, it seems a matter of no importance.

I should have been glad to have had the opportunity of examining more than these three typhoid cases; but there is not the least doubt that the result would have been always the same in cases of *true* typhoid fever, (*Abdominaltyphus* of

(1) The temperature rose on a few days up to somewhat more than 25° C. (77° F.)

the Germans), and provided the patients had succumbed before the ulcerations of the small intestine had completely disappeared, or before any such ulcerations were at all met with. The latter phenomenon, I am told, is often observed in severe epidemics, when the sick are carried off very rapidly. In a publication of recent date on this subject, Fraenkel and Simmonds say (*Zeitschr. f. Hygiene*, Bd. II., Heft 1, 1887, p. 138) that they have now come to look upon the results of the bacteriological examination of the abdominal viscera (spleen) as conclusive in all cases where the macroscopical features of the abdominal organs are insufficient to secure the anatomical diagnosis. They furnish a characteristic instance where the clinical observation admitted of a diagnosis other than typhoid, and also the result of the *post mortem* was in no way decisive until the disclosure of typhoid-bacilli put an end to every doubt. They give also as instance a striking illustration of a case which clinically looked very much like typhoid, and for which the *post mortem* failed to allow an undoubted answer as to its nature, till consequent upon the absence of colonies of typhoid-bacilli in gelatine-plates sown with spleen-pulp, this answer could be given in a negative sense.

I now wish to say some words about the results of experiments made on dejecta from typhoid-patients with the view of finding, and isolating the typhoid-bacilli. I have carried out a good number of such experiments; the evacuations coming from patients in different stages of the disease were examined quite fresh. Everybody who has made similar examinations knows that the diseased intestines contain enormous masses of bacteria, both in quantity and quality, and that for this reason only minute parts of the raw-material should be started from. I generally mixed a medium-sized platinum-loop full of the dejection with about 10 ccm. of a .6 p.c. sterilised salt solution in a test-tube, thence preparing two attenuations in nutritive gelatine by taking about three platinum-loops each time. The contents of the second gelatine-tube afterwards proved to be mostly fit for examination.



Now it will not, I think, be surprising to hear that among the different colonies—they developed in a comparatively short time (see above)—such as belong to typhoid-bacilli could not always be detected. It must be remembered that, first, a plentiful occurrence of typhoid-bacilli in the contents of the small intestine, and consequently in the fæces, depends on a certain phase in the course of the disease; that, secondly, the data of the patients with regard to the beginning of the fever are not always quite reliable; and that, thirdly, after what Dr. Peirce was good enough to tell me, perhaps not all the cases under treatment, which furnished me with material, might have had to do with typhoid proper. I myself witnessed in the above Hospital a *post mortem* examination made by Dr. Young on a man who had been sent to that institution as suffering from pneumonia, presumably secondary to typhoid-fever; the ileum, however, failed to show any traces of there having lately existed alterations of a typhoid character. In gelatine and agar-agar sown with pulp of spleen grew two kinds of colonies of micrococci which were not further examined.

As already indicated in several cultivations, especially when the disease was in middle stages, colonies in more or less considerable numbers were found which actually proved to be the bacillus of typhoid fever. I may abstain here, just as I did above, from entering into a detailed description of the characters exhibited by the bacilli under cultivation in the different nutrient media, etc.; those who are more especially interested in the matter will find every information in Flüggé's *Microorganismen*, Leipzig, 1886. What is besides necessary to know about variations in their mode of growth on boiled potatoes has been dealt with above (pp. 205-207).

Time did not permit me to study the behaviour of the other bacteria from the dejecta more than was required for the intended isolation of the typhoid-bacillus. As a rule such colonies as were liquefying the gelatine were quantitatively very few in comparison with non-liquefying ones. Among the latter group,

perhaps the commonest of all were colonies of bacilli which bore a certain resemblance to those of the typhoid-bacilli, and which are probably the same as *Bacillus Neapolitanus* (Emmerich) (1). The potato-culture each time revealed their non-identity with the *Bacillus typhi abdominalis*.

As a matter of curiosity rather than interest I may mention that I once found, on a plate of gelatine, a non-liquefying colony of slender bacilli which grew in the shape of a beautiful, greyish network of delicate, much elongated ramifications; such colonies bear a strong resemblance to those of *Micrococcus viticulosus* (Flügge-Microorganismen 1886, p. 178); the mode of growth on an inclined surface of nutrient gelatine is also similar.

There was no opportunity for me to extend the search for typhoid-bacilli to blood from typhoid-patients. During my stay at the Hospital no well-marked cases of roseola-formation having occurred, I preferred to leave this kind of examination in suspenso. However, I tried some blood from a roseola-like spot with one patient, but without success.

My wish to obtain some exact data as to the disinfecting and destroying powers of the commonly used disinfectants for typhoid dejections—carbolic acid, carbolised chalk, sulphate of iron, and some others; and further to ascertain how the typhoid germs in such dejections are acted upon by pure lime and slaked lime, the efficiency of which substances on cultures of cholera-spirilla and typhoid-bacilli has lately been experimentally proved by Liborius (*Zeitschrift f. Hygiene*, Bd. II., Heft 1, 1887, pp. 15-51), has equally to be put off to some later date.

(1) Flügge, l.c., p. 270-272. It is much to be regretted that a pure-culture of this bacillus sent to me by Professor Flügge, with other cultures, did not survive the voyage. It is to this microbe that Emmerich attributes or attributed the cause of Asiatic-cholera. According to other observers, however, this microbe is a common appearance in the contents of the intestines of man and animals.

Before concluding this report I may as well point out in a few words, that I commenced to go to see the leprosy-patients in the Asylum at Little Bay. In blood taken from three of them—two Chinamen, one native of Java—from spots which were free from leprosy maculæ or tumours, no bacilli were found, whereas in blood or serum derived from tubercles cut across, bacilli of leprosy, either single or aggregated in dense masses or bundles, were abundant. I hope to be able by-and-by to contribute a little to the knowledge of the biology of this interesting micro-parasite.